Human Growth Hormone

Edited by

A STUART MASON
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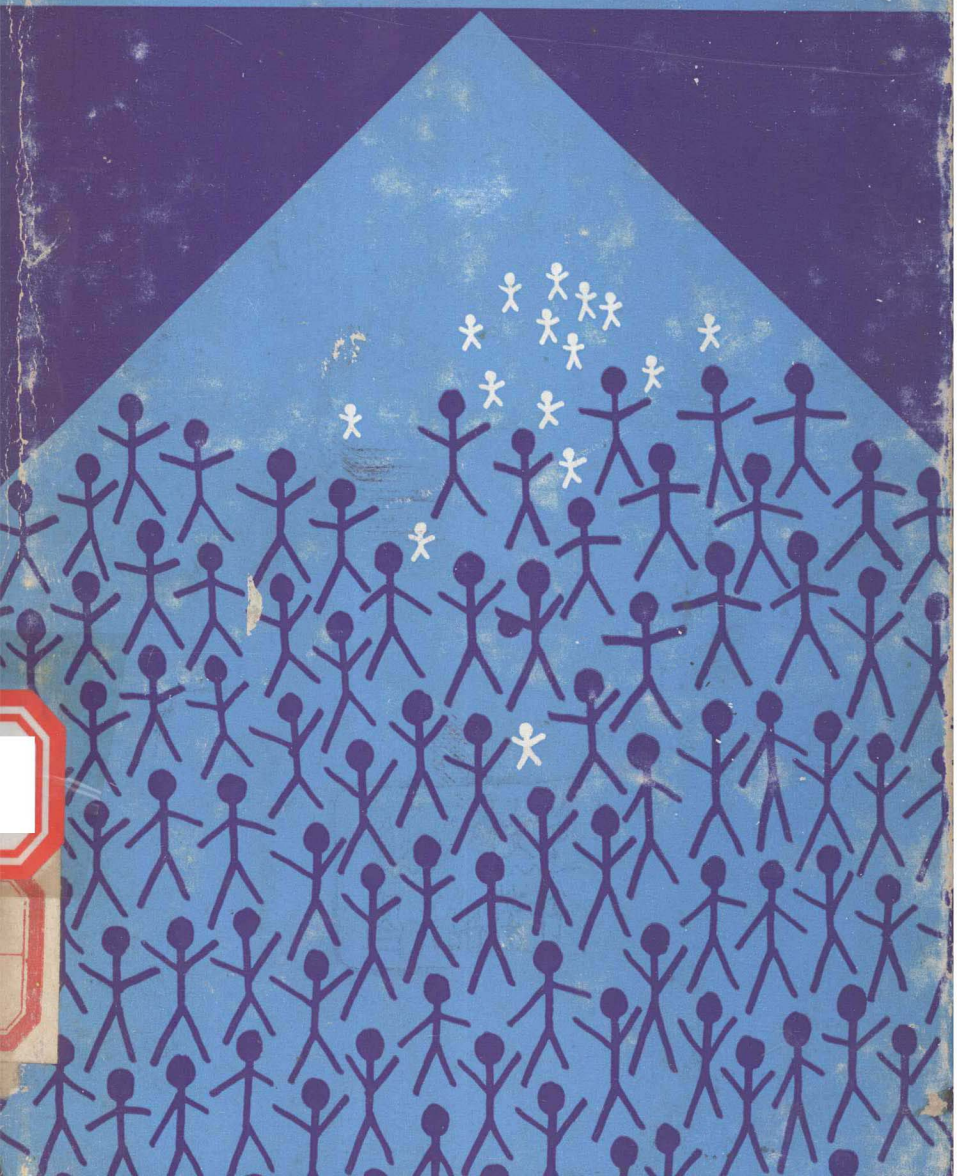

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A. STUART MASON, M.D., F.R.C.P.

WILLIAM HEINEMANN MEDICAL BOOKS LIMITED

First Published 1972

ISBN 0 433 20340 4

Printed and bound in Great Britain by R. J. Acford Ltd., Industrial Estate, Chichester, Sussex

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Preface

Fifty years ago Evans and Lond demonstrated the growth promoting activity of a pituitary extract. Their findings confirmed earlier suspicions that the anterior pituitary had something to do with statural growth and aroused exciting therapeutic possibilities. These hopes remained unrealised despite increasing knowledge of animal growth hormones. However the clinical situation was completely transformed by the relatively late discovery that growth hormones were species specific. From this discovery came the successful treatment of hypopituitary dwarfism with human growth hormone and the measurement of plasma levels of the hormone by immuno-assay allowing the clinician to detect the growth hormone deficient patient.

The volume and diversity of recent research on human growth hormone make it impossible to present a comprehensive and coherent review of the whole subject. Therefore the theme of this monograph is a practical one. It deals with all aspects of human growth hormone that are relevant to the treatment of growth hormone deficient dwarfs, from the preparation of the hormone to its effects and interactions with other hormones and the methods of detecting growth hormone in human plasma together with the necessary clinical applications. Each contributor has advanced this field of knowledge and was asked to emphasize the practical aspects of their work, reviewing the subject in the light of their own experience. Any errors in the design of the monograph must be attributed to the editor.

More and more people are becoming concerned with problems of human growth, be they protein chemists asked to prepare the human growth hormone, clinical pathologists investigating growth failure or physicians caring for dwarfed patients. For all these experts this monograph should prove useful.

I am very grateful to the contributors who represent international endocrinology for recording their experience. I am also indebted to Dr Raymond Greene for his helpful criticism and to Mr Owen Evans and his colleagues at Heinemann's for their patient work.

A.S.M.

January, 1972

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Chapter One

Preparation and Properties of Human Growth Hormone

ANNE STOCKELL HARTREE

Because of the relative scarcity of human pituitary glands and the requirement of comparatively large amounts of human growth hormone (HGH) for long-term treatment of patients, the demand for hormone far exceeds the supply. This has resulted in the organization of human pituitary collections in several countries and development of efficient fractionation procedures by which both growth hormone and other pituitary hormones can be isolated.

METHODS OF STORAGE OF HUMAN PITUITARY GLANDS

In order to obtain growth hormone in high yield and, as far as possible, undamaged by the activities of enzymes or bacteria, human pituitary glands should be preserved either by freezing or by storage in acetone immediately after removal. Because of its greater convenience preservation in acetone is the more commonly used method. The glands can accumulate in a stoppered bottle containing a large excess of acetone, at least 20 ml per gland. Although storage in acetone at room temperature is satisfactory for a few weeks or months, long-term storage should be at refrigerator temperatures. The acetone penetrates the tissues, removes water and fat, and also acts as an antibacterial and antiviral agent. If, as is often the case, the glands must be shipped elsewhere for hormone extraction, excess acetone is removed and the glands packed in a sealed container with only sufficient acetone to cover them while in transit. Shipping of frozen glands is more difficult since it is essential that they remain frozen until they reach their destination. For greater convenience acetone-preserved glands can be minced and dried for storage and transport in powder form. Mincing is performed in a Waring blendor or electric meat mincer in the presence of acetone. The mince is then filtered with suction, washed repeatedly with cold acetone and spread out on a tray to air-dry. It should be periodically turned with a spatula and all lumps broken up to ensure thorough drying which is complete within 24 to 48 hours. The

powder can then be stored in sealed containers at 4°C for several years if necessary. The yield of powder is approximately 100 gm per 1000 glands.[73]

METHODS FOR BIOASSAY OF GROWTH HORMONE

Prior to the development of immunoassay techniques, measurement of growth hormone activity in pituitary fractions and in purified preparations of growth hormone was performed by bioassay, usually in the hypophysectomized rat. Although immunoassay is a more rapid and sensitive method for determination of growth hormone, confirmation that the material is active in promoting growth is frequently necessary. The generally accepted methods for quantitative assessment of response to growth hormone in the hypophysectomized rat are measurement of weight gain or increase in width of tibial cartilage. Modifications of the former assay method, originally developed by Marx, Simpson and Evans (1942), have been described by Stockell Hartree, Kovačić and Thomas (1965) and Parlow, Wilhelmi and Reichert (1965). Immature female albino rats weighing 100 to 200 gm are hypophysectomized and can be used for assay two weeks later. Daily subcutaneous injection of 0·002 to 0·032 i.u. HGH for eight days results in weight gain proportional to the logarithm of the dose given. Injection of hormone for longer periods, however, gives rise to production of HGH antibodies and decreased responsiveness to the hormone.[52] For the tibia width assay[22,24] HGH is injected daily for four days, after which the animals are sacrificed, the tibia removed, split at the proximal end in the mid-sagittal plane and stained with silver nitrate. After exposure to light the calcified portions become dark brown, and the width of the uncalcified epiphyseal cartilage as measured under a low power microscope is proportional to the logarithm of the growth hormone dose. If the growth hormone potency is to be expressed in terms of international units, a standard preparation of known potency should be included in the assay. The standard and each unknown preparation should, if possible, be tested at two or more dose levels. Methods for calculation of potencies and 95% confidence limits can be found in the publications of Gaddum (1953) and Borth (1960).

METHODS FOR EXTRACTION AND PURIFICATION OF HGH FOR CLINICAL USE

In order that the most efficient use can be made of relatively scarce human pituitary material the methods developed for extraction of growth hormone often include procedures for preparation of other hormones as well. The glycoprotein hormones (follicle-stimulating hormone, FSH; luteinizing hormone, LH; and thyroid-stimulating hormone, TSH) are usually separated from growth hormone by their greater solubility in aqueous ethanol or ammonium sulphate solutions. In general, three types

of initial extraction media have been used for HGH; either hot glacial acetic acid, alkaline solution above pH 10 at 4°C or aqueous buffer near neutral pH. The latter, comparatively mild extraction conditions, are effective in preparing growth hormone from frozen glands but result in incomplete extraction and low yields when applied to acetone-dried pituitaries. The more drastic conditions necessary for extraction of HGH from acetone-dried glands may inactivate the relatively labile glycoprotein hormones. If they are also to be recovered, a preliminary extraction of these hormones can be performed under conditions where HGH is insoluble.

The aim of most methods for preparation of clinical grade growth hormone is to produce a high yield of active hormone on a fairly large scale, but not necessarily to obtain a homogeneous preparation. Although there have been a number of methods developed for growth hormone extraction, only those which are used for preparing clinical grade HGH of potency approximately 1·0 i.u./mg will be discussed here. In order to compare yields obtained by different methods it is assumed that a fresh pituitary gland weighs 0·67 gm and yields 0·10 gm of acetone-dried powder.

A. **Method of Roos, Fevold and Gemzell (1963)**

In this procedure approximately 300 frozen pituitary glands are homogenized and extracted in dilute phosphate buffer at pH 6·2. The soluble extract is adjusted to pH 7 and HGH is precipitated by addition of ammonium sulphate to approximately 2 M. After centrifugation the supernatant solution containing LH and FSH activities is saved and the precipitate further purified by gel filtration on Sephadex G-100 to yield clinical grade growth hormone, about 3 mg per gland.[66]

B. **Method of Li (1957, 1960)**

Freeze-dried human pituitary glands are extracted with a solution of calcium oxide at pH 10·5 and crude growth hormone is precipitated with 1·9 M ammonium sulphate. The supernatant solution containing FSH is saved and the growth hormone precipitate further purified by chromatography on IRC-50. A side-fraction containing LH is recovered in this step and the growth hormone-containing fraction further purified by ammonium sulphate precipitation and ethanol fractionation to yield 7·7 mg of clinical grade hormone per gland. Up to 1000 glands can be extracted in one batch.

C. **Method of Wallace and Ferguson (1961)**

This method is applicable to small batches of about 10 frozen human pituitaries which are thawed and extracted with sodium borate buffer at

pH 8·6. The soluble extract is chromatographed on a column of DEAE-cellulose at 4°C with a gradient in pH from 8·6 to 7·2. Clinical grade HGH is eluted in a fraction well-separated from other components in a yield of 11·2 mg/gland. The product also contains significant amounts of LH activity.

D. **Method of Lewis, Cheever and Seavey (1969a)**

Frozen pituitaries (up to 20 per batch) are thawed and extracted with 0·3 M KCl at pH 5. The soluble extract contains some crude HGH which is precipitated at pH 4·5, the remaining solution being saved for recovery of glycoprotein hormones. Additional crude growth hormone is extracted from the pituitary residue at pH 9, combined with the crude HGH obtained in the previous step and the solution fractionated and concentrated by ultrafiltration through membranes. An ultrafiltrate that passes an XM-50 membrane (permitting passage of molecules less than 50,000 M.W.) is dialyzed and freeze-dried to yield 8 mg/gland of potency somewhat greater than the usual clinical grade growth hormone.

E. **Method of Wilhelmi (1961), and Parlow *et al.* (1965)**

With this method up to 1000 pituitaries, either frozen or acetone-preserved, can be processed in one batch. An initial extraction of glycoprotein hormones is performed with 1·25 M ammonium sulphate solution at pH 4. Growth hormone is then extracted from the pituitary residue with water, adjusted to pH 9, and further purified by fractional precipitation with ammonium sulphate at pH 7. It is reprecipitated with ammonium sulphate at pH 4 and fractionally precipitated with ammonium sulphate once again to yield 3·2 mg of HGH per gland.

F. **Method of Raben (1959)**

Acetone-dried pituitaries (up to 2000 per batch) are extracted with glacial acetic acid heated briefly to 70°C. The soluble extract is treated with sodium chloride solution and acetone, and the precipitate which forms is removed. Crude growth hormone is precipitated from the supernatant solution by addition of ethyl ether, and the precipitate is dissolved in 0·1 N acetic acid and treated twice with oxycellulose to remove ACTH. The growth hormone-containing solution is made 10 N in potassium hydroxide, brought to pH 8·5 and a precipitate removed by centrifugation. The solution is treated with an equal volume of ethanol at 5°C to precipitate growth hormone in a yield of 4·4 mg per gland.

G. **Method of Reisfeld, Lewis, Brink and Steelman (1962)**

Acetone-dried pituitary powder is extracted with 0·3 M KCl at pH 5·5 and clinical grade growth hormone precipitated from the soluble extract

at pH 8·5 and −2°C by addition of ethanol to 30% by volume. Additional clinical grade HGH is precipitated by adjusting the supernatant solution to pH 5·5, and the remaining solution contains most of the glycoprotein hormones. More growth hormone is obtained by extraction of the pituitary residue at pH 10 with 0·3 M KCl. This extract, when dialyzed and freeze-dried, can be used as clinical grade HGH. The total yield of HGH is 20 mg per gland, but the potency is somewhat less than 1·0 i.u./mg and the preparation contains significant amounts of LH activity. The method is applicable to batches of up to 1000 glands.

H. Method of Elrick, Yearwood-Drayton, Arai, Leaver and Morris (1963)

Acetone-dried pituitaries (10 to 100 glands per batch) are extracted with 0·3 M KCl at pH 5·5. The soluble extract, which contains FSH, LH, TSH and ATCH, is removed by centrifugation and the residue is extracted with 0·1 N NaOH at room temperature. After centrifugation clinical grade HGH is recovered from the supernatant by dialysis and lyophilization in a yield of 29 mg per gland. Some of the assays for activity were by immunoassay and the reported potencies of 1·0 i.u./mg for clinical grade HGH and 0·4 i.u./mg for crude pituitary powder may not be accurate estimates of biological activity. The latter potency is several times higher than has been observed by other workers.

I. Method of Mills, Ashworth, Wilhelmi and Stockell Hartree (1969)

This method, developed for large-scale use (up to 2000 glands per batch) with acetone-dried glands, is also effective in extracting HGH from embalmed pituitaries. It is a modification of the original method of Wilhelmi (1961) with fewer steps, and it includes an initial extraction with 0·1 N sodium hydroxide for more complete extraction of HGH[14] and an oxycellulose treatment to remove ACTH.[63] Preliminary extraction of glycoprotein hormones, with 6% ammonium acetate in 40% ethanol, permits recovery of these hormones and results in clinical grade HGH with negligible contamination by other hormone activities. The yield is 7·2 mg per gland, but is lower if embalmed glands are used (2·3 mg per gland). Full details of this method are given in the appendix.

POINTS TO CONSIDER IN CHOOSING A METHOD

Some patients treated with HGH have developed antibodies to the hormone.[19,57,62,68,75,78] Although in many cases the antibody titres were low and did not appear to affect the growth response to the hormone, there were a significant number of patients who developed high antibody titres and became unresponsive to further therapy with HGH. Since most

patients who developed resistance to HGH therapy had been treated with hormone prepared by the method of Raben (1959), it was postulated that the rather drastic extraction conditions employed in the method might have altered the protein sufficiently to render it antigenic in some patients. If this is the correct explanation for the antigenicity of the preparation, extraction of HGH under relatively mild conditions may be an advantage. However, one case of resistance to HGH therapy associated with development of a high concentration of antibodies to the hormone has been reported[28] where HGH extracted by the mild procedure of Roos (1968) was used exclusively. It was suggested that genetic factors might influence the development of antibodies in this patient since three other patients related to this one, but treated with Raben HGH, also developed high antibody titres to the hormone.

The procedure of Lewis *et al.* (1969a) has been designed to extract high potency hormone under conditions where denaturation and aggregation of HGH are minimized, and the product may be less likely to elicit the production of antibodies to the hormone. In cases where frozen glands are available and relatively small numbers need to be extracted, this procedure should be extremely useful and the yield of hormone is high. The methods of Wallace and Ferguson (1961), Reisfeld *et al.* (1962) and Elrick *et al.* (1963) give good yields of HGH by weight, but the products contain significant amounts of other hormone activities. When large numbers of acetone-preserved glands are to be processed, the method of Mills *et al.* (1969) can be recommended. It is a comparatively mild extraction procedure adaptable to large-scale work and the product, obtained in good yield, is free of significant contamination by other hormone activities except for prolactin. The relationship between human growth hormone and human prolactin is discussed in a later section.

It has been reported that more HGH can be extracted from frozen human pituitaries than from acetone-preserved glands.[56] However, the yield of HGH obtained by these workers from frozen glands (between 7 and 8 i.u. per gland) is no greater than the yield from acetone preserved glands obtained by Mills *et al.* (1969). It seems likely that the saline solution at pH 8, used for extraction by Oliner *et al.* (1968), is satisfactory only for frozen material and that a more alkaline pH is required to dissolve the HGH present in acetone-dried pituitaries.

PROCEDURES FOR AMPOULING HGH FOR CLINICAL USE

For clinical use HGH must be sterilized and dried in such a way that it can easily be dissolved for injection. Sterilization by heating is unsatisfactory since it results in denaturation of the protein hormone with loss of biological activity. Filtration through a membrane filter is a satisfactory

procedure for sterilization, but there are several problems involved in carrying it out. The following suggestions for preparing the material for ampouling are based on helpful discussions and advice received from Dr. A. E. Wilhelmi, Emory University, U.S.A. and Dr. A. W. Phillips, Wellcome Research Laboratories, England.

There may be initial difficulty in dissolving HGH, particularly if it was previously dried by organic solvents. Therefore the hormone is mixed first with dilute alkali (10 ml of 0·05 N NaOH per gram of protein) followed by distilled water (approximately 40 ml per gram of protein). This brings the bulk of the material into solution and the pH is approximately 8·5 to 9. Pituitary proteinases active at pH 7·0 to 8·5 are frequently present as contaminants of clinical grade HGH[35] and can cause significant inactivation of the hormone in solution during the sterile filtration and ampouling procedures. Therefore it is advisable to cautiously lower the pH to approximately 6·8 by addition of 0·5 N HCl slowly with stirring, and to work at 4°C, in order to minimize proteolysis during the procedure. If the pH is brought too low the hormone will precipitate from solution. After pH adjustment, water and mannitol (4 gm per gm of HGH) are added to bring the solution to the appropriate concentration for ampouling (approximately 100 ml of solution per gram of HGH). The mannitol serves as an inert carrier which protects the protein from denaturation and improves the solubility of the freeze-dried product. The solution should then be centrifuged at 4°C to remove insoluble matter and passed through a membrane filter for sterilization. It may be more convenient to use a 0·45 micron filter first for removal of any remaining particulate matter, but a 0·22 micron filter under sterile conditions is required for adequate sterilization. If necessary the solution can be stored overnight in a sterile bottle at 4°C. It is then aliquotted into ampoules, freeze-dried, and the ampoules sealed. The ampoules should be visually inspected, examined for holes, and tested for sterility and pyrogens. After the techniques of dissolving and sterile filtration have been mastered, it is most convenient to ampoule a large batch of HGH at one time since there may be some variation between batches in potency of the final product. Representative ampoules from each batch should be assayed for biological activity, and the ampouled hormone is stable for long periods of time stored at 4°C.

PHYSICOCHEMICAL AND IMMUNOLOGICAL PROPERTIES OF HUMAN GROWTH HORMONE

Clinical grade HGH made by any of a number of methods has been shown to be heterogeneous by gel electrophoresis and usually contains from three to five electrophoretic components.[3,16,31,35] Kaplan and Grumbach (1962) showed that the three major components of HGH

prepared by Raben's method have similar and possibly identical antigenic determinants. Thus they are likely to be closely related species of the same protein molecule. Hunter (1965) reported that gel filtration on Sephadex G-200 of HGH prepared by Raben's method yielded two peaks of material absorbing at 280 mμ. The first peak which emerged near the exclusion volume of the column, and therefore would be assumed to have a molecular weight of 100,000 or more, was low in growth promoting activity and in immunologically active HGH. The second peak, with elution volume corresponding to protein of 20,000 to 30,000 molecular weight, contained the major portion of the growth promoting activity and immunologically active HGH. The immunological properties of the high molecular weight component suggested that it consisted mainly of aggregated, possibly denatured, growth hormone.

Wilhelmi and co-workers[77] have purified their clinical grade HGH by chromatography on DEAE-cellulose with gradient elution from 0·05 M to 0·2 M sodium chloride solution at pH 8. Two fractions of high biological activity (approximately 2·0 i.u. per mg) but with different electrophoretic mobilities were obtained. Some of the heterogeneity of clinical grade HGH is believed to result from loss of one or more amide groups by enzymatic action or exposure to alkali during the extraction procedure.[35,36,77] Freeze-drying of HGH preparations has also resulted in the appearance of new electrophoretic components[38] and changes in the gel filtration pattern of the hormone.[25]

Berson and Yalow (1966) reported that storage of HGH in dilute acid at −15°C or in acid, neutral or alkaline solution at 20°C resulted in appearance of more acidic components on gel electrophoresis.

Purification of the electrophoretic components of HGH prepared by the method of Roos *et al.* (1963) has been achieved by Peckham (1967). A proteolytic enzyme inhibitor (di-isopropylfluorophosphate) was added to prevent enzymatic degradation and the hormone was chromatographed on a column of Sephadex A-50 at −4°C in 0·05 M phosphate, pH 6·8, containing 15% ethylene glycol. Under these conditions the HGH was separated into two distinct electrophoretic components, each with growth promoting activity of 2·0 i.u./mg.

RELATIONSHIP BETWEEN HUMAN GROWTH HORMONE AND HUMAN PROLACTIN

It has been repeatedly observed that purified preparations of HGH possess considerable prolactin activity as determined by pigeon crop sac-stimulating, lactogenic or luteotrophic assay.[9,15,33] Likewise, purified preparations of human prolactin contain appreciable growth-promoting activity.[2,47,81] Although there have been several reports of wide variations in the ratio of growth promoting to prolactin activity in purified hormone preparations,[2,72,77] complete separation of the two activities from human

pituitaries has not been achieved. Tashjian *et al.* (1965b) reported that the two major electrophoretic components of HGH had very different prolactin activities but nearly identical growth promoting potency. On the other hand, Peckham, Hotchkiss, Knobil and Nicoll (1968) found each component of HGH to have virtually the same prolactin as well as growth promoting potency. It seems likely that both activities are present in the same protein molecule although it is possible that each resides in a different portion of the molecule. This is not true of a number of other mammalian species, including beef and sheep, in which growth hormone and prolactin activities are found in distinct and separable protein hormones.[48] In the human, normal lactation has been observed in subjects who have an isolated deficiency of HGH as judged by dwarfism and low or undetectable concentration of HGH in plasma, but who have normal levels of other trophic hormones.[65] Therefore it appears that HGH is not required in the human for post-partum lactation. This is further supported by the studies of Frantz and Kleinberg (1970) who measured plasma prolactin by a sensitive bioassay and found that postpartum patients and patients with galactorrhea had high levels of this activity which was not neutralized by antiserum to human growth hormone. However, Nicholson (1970) found that pituitaries from pregnant and post-partum women did not contain a higher concentration of prolactin than those of men or normal women, and that in crude pituitary extracts subjected to gel electrophoresis there was no evidence of a stainable protein which was characteristic of pregnancy and the post-partum period. Peake, McKeel, Jarett and Daughaday (1969) reported an interesting study of a human pituitary tumour that contained significant prolactin activity as measured by stimulation of the pigeon crop gland, but only negligible amounts of HGH as determined by radio-immunoassay. It is not known, however, whether the prolactin present in this tumour is a normal component of human pituitaries. As yet there is no definitive evidence for the existence in normal human pituitaries of a prolactin molecule which is separate and distinct from HGH. The presence in the gland of large amounts of HGH containing intrinsic prolactin activity increases the difficulty of obtaining such evidence.

Another human hormone possessing prolactin activity has been isolated from placental tissue. This hormone, human placental lactogen, cross-reacts immunologically with HGH, but is very low in growth promoting activity.[17,20,29] There are structural similarities between human placental lactogen and HGH which will be discussed in a later section.

MOLECULAR WEIGHTS OF GROWTH HORMONES FROM VARIOUS SPECIES

Large differences have been reported between the molecular weights of pituitary growth hormones obtained from various species,[12,43] and it has

been suggested that molecular size may be of considerable importance in determining the species specificity of the hormone.[39] However, it has become apparent from more recent determinations of molecular weights in dilute solutions or in dissociating solvents, that growth hormones from beef, pig, sheep, human and monkey pituitaries have similar molecular weights, in the neighbourhood of 22,000.[1,10,82] Under the usual conditions for determining sedimentation constants in the ultracentrifuge, aggregation of growth hormone (GH) can occur, resulting in apparent molecular weights of 40,000 or more for beef GH and pig GH.[82] It is not known whether aggregation occurs under normal physiological conditions, but this seems unlikely at the low concentration of GH found in blood serum. It is of interest that pituitary prolactins from sheep or beef sources also have molecular weights in the neighbourhood of 22,000,[1,10] and that the molecular weight of human placental lactogen as determined in guanidine solution is approximately 19,000.[17]

IMMUNOLOGICAL RELATIONS BETWEEN HGH AND GROWTH HORMONES FROM OTHER SPECIES

Extensive immunological studies of GH from various species have been performed in a number of laboratories but only a summary of the main findings will be given here. In agar gel diffusion studies, antiserum to beef GH reacted with sheep GH and beef GH but not with GH from human, monkey, pig or whale sources.[26] In similar studies with antiserum to HGH, reactions with HGH and monkey GH were demonstrated, but there was no cross-reaction with sheep GH or beef GH.[42] However, after digestion of bovine GH with pepsin, a fragment was released which showed a weak immunological cross-reaction with HGH in a haemagglutination system.[34] Josimovich and MacLaren (1962) showed that human placental lactogen cross-reacted immunologically with HGH in an agar gel diffusion system using antiserum to HGH. In further studies Josimovich and Mintz (1968) observed immunological cross-reactions between HGH, monkey GH, human placental lactogen and monkey placental lactogen which suggested that the four hormones shared a common antigenic determinant.

The more sensitive immunological technique of quantitative micro-complement fixation was used by Tashjian, Levine and Wilhelmi (1965a) to study immunological relations between growth hormones from various species. These workers showed that the degree of relatedness of non-human primate growth hormones to HGH corresponds well with the classification of primate phylogeny based on evolutionary data. For example growth hormones from the chimpanzee and the orangutan cross-reacted more strongly with HGH than did rhesus monkey GH, and squirrel monkey GH was still more distantly related. Much weaker, but demonstrable cross-reactions were observed by this technique between antiserum to HGH and

pig GH, beef GH or sheep GH. It was concluded, therefore, that some similarities in structure exist between growth hormones of all species tested, but that HGH is far more similar in structure to growth hormones from non-human primates than to other mammalian species. These same techniques were used by Tashjian *et al.* (1965b) for comparisons between HGH preparations which were relatively high or low in prolactin activity and human placental lactogen. They concluded that the two types of HGH were immunologically similar but not identical and that human placental lactogen was immunologically related to both types of HGH.

BIOLOGICAL SPECIFICITY OF HGH

Published data on the biological responsiveness of various experimental animals to growth hormones of different species have been summarized by Li and Liu (1964). It is of interest that all mammalian growth hormones studied thus far will stimulate growth in the hypophysectomized rat but the hypophysectomized guinea pig does not respond to GH from any species.[32] Although the rat responds to daily injections of non-primate mammalian growth hormones for long periods of time, the response to primate growth hormones is limited to about 10 days.[43] After this time antibodies to the hormone develop[52] and the animals become resistant to further treatment with primate growth hormones, but they are still capable of responding to other mammalian growth hormones. Both HGH and monkey GH are effective in primates, but other mammalian growth hormones are not. It has been suggested[40] that all mammalian growth hormones possess the same biologically active "core", the remainder of the protein being different for each species. This would imply that the rat can degrade a large number of mammalian growth hormones to the active "core", but that primates are unable to do so. It is because of the unresponsiveness of humans to growth hormones obtained from beef, pig or sheep pituitaries that arrangements must be made for collection and preservation of human pituitaries obtained at autopsy, so that HGH can be extracted for clinical use.

EVIDENCE FOR RETENTION OF BIOLOGICAL ACTIVITY AFTER PARTIAL DIGESTION OF GROWTH HORMONE WITH PROTEOLYTIC ENZYMES

For a number of years efforts have been made in several laboratories to obtain a biologically active "core" by digestion of beef GH with proteolytic enzymes. It was hoped that a preparation of this type might be active in the human and thus make available for clinical use larger quantities of the hormone than can be obtained from human pituitaries. Limited digestion of beef GH, human GH or monkey GH with chymotrypsin has been performed without loss of biological activity.[40] Other proteolytic enzymes